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(71) Applicant: WISCONSIN ALUMNI RESEARCH FOUNDATION [US/US]; P.O. Box 3765, Madison, WI 53707-7365 (US).

(72) Inventor: DEUTSCH, Harold, F. ; 6401 Purcell Road, Belleville, WI 53508 (US).

(74) Agents: HIERL, Michael, A. et al.; Olson & Hierl, Ltd., 20 North Wacker Drive, Suite 3000, Chicago, IL 60606 (US).

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(54) Title: COMPLEXES OF ANTHRACYCLINE ANTIBIOTICS WITH POLYUNSATURATED FATTY ACIDS IN LIPID EMULSIONS

(57) Abstract

Compositions are provided wherein a complex of an anthracycline antibiotic with a polyunsaturated fatty acid (with or without α-fetoprotein) is dissolved in a lipid emulsion. Dissolved uncomplexed α-fetoprotein can also be present. The compositions are useful in a method for the intravascular treatment of neoplastic tissue, especially α-fetoprotein producing hepatoma cells in humans, at high doses of the complex for relatively short periods of time.

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COMPLEXES OF ANTHRACYCLINE ANTIBIOTICS WITH POLYUNSATURATED FATTY ACIDS IN LIPID EMULSIONS

Related Application

This application is a continuation-in-part of my pending application Serial No. 765,550, filed September 25, 1991.

Field of the Invention

The present invention relates to solutions of complexes of anthracycline antibiotics with polyunsaturated fatty acids in lipid emulsions, and to the use of the solutions alone or in combination with alpha-fetoprotein for the in vivo treatment of neoplastic tissue.

Background of the Invention

Deutsch et al. in "Cytotoxic Effects of Daunomycin-Fatty Acid Complexes on Rat Hepatoma Cells" published in Cancer Research, 43, 2668-2672 (1983) describe two component complexes of daunomycin and polyunsaturated fatty acids (PUFAs) and their utility for treating rats with liver tumors that secrete alpha-fetoprotein (AFP). Rat serum was employed as a carrier, but was found to dissolve only about 50 to 100 μg/ml of such a daunomycin/AA complex.

Also, in Tumor Biology, 8, 289-290 (1987), Deutsch et al. describe complexes of an anthracycline antibiotic (AA) with both a PUFA and AFP and their uptake by tumor cells from serum albumin solution.

In adult mammals, including especially man, circulating AFP which originates from alpha-fetoprotein-producing neoplastic tissue (particularly hepatomas) binds to PUFAs in the blood and the resulting AFP/PUFA complexes circulate in the blood back to the liver and hepatomas

located therein. The AFP also is preferentially or selectively absorbed by many different types of neoplastic tissue including both tumor cells which produce AFP and tumor cells which do not (see, for example, H.F. Deutsch, "Chemistry and Biology of α-Fetoprotein" in Advances in Cancer Research, 56, 253-312 (1991).

As described by Deutsch et al. in the foregoing Tumor Biology article, daunomycin or another anthracycline antibiotic (AA) that has been preliminarily bound to a PUFA (before administration to a patient) is delivered as a part of an AFP/PUFA complex into the tumor cells and displays the desired cytotoxic activity. Compared to free AA, the AA/PUFA complexes and the AA/PUFA/AFP complexes display substantially reduced cytotoxicity with regard to other (normal) body tissue (Deutsch et al. in Cancer Res., ibid.).

However, as Deutsch et al. also observe in the foregoing papers, daunomycin/PUFA complexes (whether or not further complexed with AFP) have very low solubilities in water and aqueous media, such as normal rat serum, normal human serum albumin, and the like. Also, the low binding affinity of a protein such as serum albumin for such a complex may present a problem in administration. In addition, albumin has a tendency to bond to saturated fatty acids so that a complex of albumin with a fatty acid and an AA could lead to the undesired targeting of AA to normal cellular tissue. Thus, in the prior art, no suitable aqueous carrier for achieving a high dose level for the daunomycin/PUFA complex is known.

It would be desirable to intravascularly administer to a mammal (especially man) a relatively large dose of a daunomycin/PUFA complex in a substantially dissolved form in a single relatively small volume solution. This would provide the capability of achieving a

concentrated dose over a short administration time interval so that significant antitumor activity against neoplastic and other tumor tissue could be achieved through complexing with serum AFP, thereby to achieve substantial destruction of tumor cells without injuring the normal cells of the body over a relatively short time interval.

Only a concentrated solution of a complex of AA with PUFA (whether or not AFP is additionally complexed therewith) now appears to offer the capability for achieving safe and useful high dose rates of AAs. For example, the use of a complex of an anthracycline antibiotic (such a daunomycin or adriamycin) with antibodies was disclosed by Sela et al. in U.S. Patent No. 4,263,279. However, the antibody is a foreign protein which can not be repeatedly administered because of the antibody response of the recipient. Thus, high dose rates for short terms are not practical with such an anthracycline antibiotic/antibody complex. Also, there is a question of whether or not these antibody complexes can enter the cells of the neoplastic tissue to be treated.

However, in order for an AA/PUFA complex (whether or not such is further complexed with AFP) to be suitable for effective intravascular administration at a high dose rate over a relatively short time period for the treatment of mammalian (including human) neoplastic tissue, a liquid carrier is needed in which the complex is relatively highly soluble. In addition, this carrier must also be physiologically compatible with blood and solid tissue, and must be non-toxic. Such a carrier/complex composition has not previously been known.

Also, to target an AA/PUFA complex to tumor cells when there is little or no circulating serum AFP present would mean that, even at the desired high dosage rate

(assuming that such were achievable), little of an AA/PUFA complex would reach and be absorbed by tumor cells.

Clearly, there is a need for new and improved compositions and methods for treating neoplastic tissue with AAs.

Summary of the Invention

More particularly, this invention is directed to compositions comprised of solutions of (a) complexes of anthracycline antibiotics with polyunsaturated fatty acids and optionally with alpha-fetoprotein in a lipid emulsion and (b) alpha-fetoprotein in water (preferred) or a lipid emulsion.

This invention is further directed to improved in vivo methods for treating mammalian neoplastic tissue which methods utilize such compositions. These methods involve the intravascular administration, preferably intravenously, of a composition of this invention, either alone or in combination with solubilized alpha-fetoprotein, to a mammalian patient who is afflicted with a neoplasm, particularly a neoplasm which absorbs AFP. It is presently preferred to practice the method of this invention by so administering to such a patient a composition of this invention that includes an AA/PUFA complex in a lipid emulsion (LE) concurrently with an aqueous solution of AFP.

The alpha-fetoprotein has a high affinity for polyunsaturated fatty acids and is able to combine with these fatty acids even when they are themselves bound to anthracycline antibiotics. Because alpha-fetoprotein is taken up by a wide variety of tumor cells, and because it is able to carry the fatty acids bound to it into such cells, complexes of alpha-fetoprotein with fatty acids which are also bound to anthracycline antibiotics are taken up by

tumor cells. In these resulting cells, the cytotoxic properties of the drug are operative. The effect is to selectively destroy tumor cells.

The methods of this invention are thus very useful not only for treating tumor cells of the types which produce, release and absorb alpha-fetoprotein, such as, for example, hepatoma cells, but also for treating tumor cells of the types which do not produce alpha-fetoprotein but which absorb alpha-fetoprotein, such as, for example, the peripheral lymphoid cells found in leukemia patients.

The compositions and methods of this invention are particularly surprising and effective for the treatment of such tumor cells because of the surprising and unusual characteristics of the compositions of this invention. For one thing, the compositions can contain, if desired and as is presently preferred, relatively high amounts of a dissolved complex of an anthracycline antibiotic (AA) and a polyunsaturated fatty acid (PUFA) with AFP optionally also being complexed therewith. Thus, such compositions make possible the administration of a relatively concentrated dose of a complex of AA with PUFA (with incorporated AFP being optional) in a single, relatively small volume solution over a short administration time interval.

For another thing, such compositions can be administered intravascularly to a mammal (including man) without adverse side effects and with low toxicity particularly over relatively short time intervals. For example, a present preference is to employ in such a composition an amount of about 60 to about 150 milligrams (mg) of dissolved complex per milliliter (ml) of lipid emulsion (based on AA/PUFA complex independently of whether or not AFP has been complexed therewith) in each 100 milliliters (ml) of the lipid emulsion.

The lipid emulsion is a liquid carrier which is itself characteristically substantially non-toxic and which permits intravascular administration of a composition of this invention into a mammal (particularly man) without significant adverse or undesired side effects. An AA/PUFA complex (whether or not the complex includes AFP) leaves the lipid emulsion after administration of the composition. The lipid emulsion apparently functions as a transfer agent which permits transfer of an AA/PUFA complex to AFP after administration into vascular fluids.

Thus, the inventive compositions and methods provide the capability of achieving safe and effective high intravascular dose rates of an AA/PUFA complex in a mammal.

The inventive compositions make possible the practice of the method of this invention by which a relatively large and therapeutically effective dose level of a dissolved AA/PUFA complex (whether or not the complex includes AFP) is administered intravascularly as a small volume dosage into the blood stream of a mammalian patient suffering from a neoplasm particularly one of the type whose cells take up AFP. Although a present preference in practicing the method of this invention is to maintain such a high dose level for a relatively short time interval, a physician can choose to periodically administer a composition of this invention at a relatively high complex dosage level to a patient if necessary or desirable for neoplasm treatment. As a result, a neoplasm can be selectively destroyed while toxic side effects to normal tissue can be minimized during the administration period.

In the case of treating a neoplasm which produces and takes up AFP, a composition of this invention offers the additional advantage of containing an active treating agent (that is, an AA/PUFA complex) which, when further complexed

with AFP in vivo, produces a targeted pharmaceutical agent for the specific treatment of such a neoplasm, such as a hepatoma. The targeting agent remains safe and effective as an intravascular treating agent.

Particularly in the case of treating a neoplasm which does not produce AFP but which takes up AFP, the method of this invention can be practiced, if desired, as a concurrent administration of the AA/PUFA complex and of AFP. For example, in one step, a composition of the invention is intravascularly administered, and in a second step, an aqueous solution of AFP is administered. Independently of the manner of the concurrent administration, the targeted three-component complex agent of AA/PUFA/AFP is formed in vivo. This complex is useful for treating AFP absorbing neoplasms (both non-AFP-producing neoplasms as well as AFP-producing neoplasms).

The invention provides an effective and safe method of treating neoplastic tissue, particularly AFP-absorbing neoplastic tissue, using a relatively concentrated solution of an AA/PUFA complex. The method is preferably practiced in combination with AFP. The method can be carried out, if desired, over a relatively short treatment period.

Other and further objects, aims, advantages, features, purposes, embodiments, and the like will be apparent to those skilled in the art from the present specification and the appended claims.

Detailed Description

(a) The Anthracycline Antibiotic (AA)

AAs reportedly bind to DNA and inhibit both DNA-directed DNA synthesis and DNA-directed RNA synthesis. Certain enzyme interactions evidently also occur. The

antibiotics of this anthracycline family are known to display similar useful neoplasm treatment capacity, but characteristically have undesirable normal cell cytotoxicity. Also, AAs are known to cause cardiac toxicity, bone marrow suppression, alopecia, tissue irritation and undesirable gastrointestinal tract symptoms.

An AA characteristically comprises a tetracycline aglycone which is bonded through an ether linkage to a basic amino sugar.

Included among the known and available AAs are daunorubicin (also known as daunomycin), doxorubicin (also known as adriamycin), idarubicin and pirarubicin. The first two are isolated from Streptomyces peucetius, while the last are synthetic analogs of adriamycin.

(b) The Polyunsaturated Fatty Acid (PUFA)

PUFAs which display high affinity for, and the ability to bind to, circulating AFP are useful in the practice of this invention. Such acids usually contain (a) a single terminal carboxyl group per molecule, (b) 18 through 24 carbon atoms per molecule (usually an even number of carbon atoms per molecule) distributed in an unbranched aliphatic hydrocarbon chain, and (c) at least three double bonds per molecule each located between a different respective pair of adjacent carbon atoms in the hydrocarbon chain.

Presently preferred PUFAs contain at least 20 carbon atoms per molecule and also at least four double bonds per molecule. Usually, the polyunsaturated fatty acid contains not more than about six double bonds per molecule. The configuration of the double bonds is cis, and the individual double bonds in each acid molecule are usually separated from one another by at least one methylene group.

Examples include the essential fatty acids linolenic and arachidonic as well as clupanodonic, docosahexaneoic, and the like. Presently preferred PUFAs are arachidonic acid ($C_{20:4}$) and docosahexanoic acid ($C_{22:6}$).

(c) The Alpha-Fetoprotein (AFP) and Aqueous Solutions Thereof

While any mammalian AFP (that is, AFP having an amino acid sequence that is characteristically mammalian) is believed to be suitable for use in this invention, it is preferred to employ for administration purposes to a given mammal species AFP that is either derived from the same species or from a phylogenetically higher species. It is presently most preferred to employ human AFP as a starting material. Human AFP can be obtained from various commercial sources. It is currently usually obtained from fetal tissues, umbilical cord blood, or from cultures of hepatoma cell lines (see, for example, Tecce et al. in Analytical Biochemistry, 168, 306-311 (1988)). Human AFP has a characteristic amino acid sequence and is a 67-kDa molecule; see, for example, Pucci et al. in Biochemistry, 30, 5061-5066 (1991).

AFP characteristically is soluble in water and aqueous physiological isotonic solutions. However, AFP displays a tendency to denature in ethanol and in lipids, and therefore is unstable in such solvents. Therefore, it is preferred to avoid administering AFP (either separately or as a preformed complex with PUFA and AA in accord with this invention from a lipid emulsion solution unless such a solution of AFP in lipid emulsion is promptly administered after its preparation.

It is presently preferred for purposes of use in the practice of this invention to prepare AFP preliminarily

as a dry powder by purification and lyophilization. Such a dry powder is then preferably dissolved in an aqueous medium preferably a physiologically compatible aqueous medium such as normal saline, before administration in accord with the teachings of this invention.

As initially obtained, AFP may be in an impure form. It can be purified by various techniques including, for example, HPLC (High Performance Liquid Chromatography) and FPLL (Fast Protein Liquid Chromatography). These methods advantageously do not significantly dilute the starting protein solution. The purified AFP is conveniently isolated with selected chromatographic column procedures using compatibly buffered eluants and then is lyophilized by a procedure such as follows.

After the AFP is recovered in purified form, salts are removed by conventional methods and solutions of AFP are placed in containers, such as glass ampoules or round bottom flasks. For greater efficiency, the solution inside each container is limited to about 20% of the useful container volume.

The solution in each container is then rapidly frozen in a cold bath, for example, a bath of acetone and solid carbon dioxide. The rapid freezing process introduces minimal shifts in the pH. A preferred technique is to hold each container at a 45 degree angle and to rotate the container slowly to allow the solution therein to freeze along the container wall in the form of a shell.

Each container containing the frozen solution is then connected to a lyophilizer. The seal to the vacuum is broken to allow maintenance of a selected vacuum in each container. A cold trap is preferably used to handle the flow of water vapor from the solvent associated with the protein.

During lyophilization, the sample is left undisturbed until all of the ice has evaporated. After this process is completed, an ampoule may be sealed under vacuum before the dried AFP is dissolved in water, isotonic salt solution or the like prior to administration.

For example, the aqueous solvent can be a 0.15 molar sodium chloride solution (approximately 0.9% salt in water) or a 0.3 molar nonelectrolyte solution. As a present preference, the concentration of AFP in such an aqueous solution for purposes of mammalian administration, in accord with the teachings of this invention, can be in the range of about 10 to about 20 mg per ml although larger and smaller concentrations can be used, if desired. A presently more preferred concentration is about 10 mg per ml.

AFP in circulating blood serum is believed to complex with PUFAs at the rate of about 1 to about 3 moles of each PUFA per mole of AFP. Complexes of AFP with PUFAs can be isolated and are useful as targeting carriers for delivery of agents complexed with PUFA to cells which take up alpha-fetoprotein. Such an agent must be complexed with the PUFA prior to the time when the PUFA is complexed with the AFP and preferably prior to agent administration to avoid the presence of the free agent in circulating serum.

(d) The AA/PUFA Complex

As described by Deutsch et al., supra, an AA is readily complexed to a PUFA by any convenient procedure. Characteristically in such a complex, a single peptide (i.e., amide) bond is now believed to exist between the amine group of the amino sugar in the AA molecule and the carboxyl group of the PUFA molecule.

For example, the complexing can be carried out under aqueous liquid phase conditions in the presence of a water soluble carbodiimide peptide coupling agent, such as

1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, dicyclohexyl carbodiimide, N,N-carbonyldiimidazole or the like. The complexing reactions can also be carried out in organic solvent media, such as chloroform or the like. Free dissolved sugar alcohols, such as mannitol which is commonly found compounded with commercially available AA, are preferably removed before the complexing reaction.

The respective starting amounts of the PUFA and of the peptide coupling agent that are employed in relation to the AA can vary. Illustratively, the starting quantity of the PUFA can be in the range of about 1.0 to about 2.0 times the AA, while the starting quantity of the peptide coupling agent can be in the range of about 4 to about 5 times the AA. Variations are possible.

The coupling reaction is conveniently carried out at ambient temperatures preferably with gentle agitation of the reaction mixture for a time interval that is preferably in the range of about 10 to about 24 hours. Thereafter, if an aqueous medium has been employed, the mixture is adjusted to a pH of about 10 to about 11 and extracted with chloroform.

Since it is desired to avoid the presence of either free AA or free PUFA in a composition of this invention, a residual small amount of either material is preferably removed at the end of the complex formation. Thus, a chloroform solution of the complex is conveniently washed at alkaline and at acid pH.

Also, the chloroform layer that results from such an extraction procedure is washed preferably several times with a dilute aqueous buffer of about pH 9. If the reaction has been carried out in an organic medium, such as chloroform, the medium is washed several times with a dilute aqueous buffer of about pH 9. The resulting solution is

then washed several times with dilute aqueous hydrochloric acid having a pH of about 3 to about 4.

The resulting chloroform solution is dried over anhydrous sodium sulfate or the like and evaporated to dryness with a rotary water aspirator or by a similar method. The product can be analyzed, if desired, by chromatography, spectrophotometry or the like. The purified AA/PUFA complex characteristically contains a mole ratio of AA to PUFA of about 1:1. This complex displays very low solubility in aqueous isotonic buffers (in a pH of about 7.0 to about 7.5) and in water. The complex is soluble in ethanol and various organic solvents.

(e) The AA/PUFA/AFP Complexes

The three component complex AA/PUFA/AFP can be prepared as indicated by Deutsch et al. in Tumor Biology, op. cit. or otherwise as desired.

A purified AA/PUFA/AFP complex is now believed to have mole ratio of AA to PUFA to AFP that is typically in the range of about 1:1:1 to about 1:1:0.5. The complex is soluble in water and physiological aqueous isotonic solutions. The complex is not stable in organic solvents such as ethanol.

(f) The Lipid Emulsion (LE)

Various lipid emulsions can be employed in the practice of this invention and such can be variously prepared as those skilled in the art will appreciate.

As reported in the American Formulary Service, which is a publication of the American Society of Hospital Pharmacists, Inc., Bethesda, MD, at page 1531 (1991), various lipid emulsions (LEs) are commercially available. Exemplary emulsions and their respective compositions are shown below in Table I wherein INTRALIPID is a trademark of KabiVitrum Ltd., Stockholm, Sweden; LIPOSYN is a trademark

of Abbott Laboratories, North Chicago, IL; and NUTRILIPID is a trademark of McGaw Laboratories, Glendale, CA. INTRALIPID lipid emulsions are available from Clintec Nutrition Co. (a joint venture of Baxter Healthcare Co., Deerfield, IL and Nestle S.A., Vevey, (Switzerland) and Kabi Pharmacia, Inc., Clayton, NC.

Other suitable commercially available lipid emulsions include products marketed under the trademarks SOYACAL by Alpha Therapeutic Corporation, Los Angeles, CA; and TRAVAMULSION by Travenol Laboratories, Inc., Deerfield, IL.

TABLE I- LIPID EMULSIONS

Preparation:	INTRALIPID® 10%	INTRALIPID® 20%	LIPOSYN® II 10%	LIPOSYN® II 20%
Manufacturer:	Clintec (or Kabi Pharmacia)		Abbott	Abbott
Components				
Total Concentrn.	10%	20%	10%	20%
Fat Content g/100 ml				
Safflower Oil	—	—	5	10
Soybean Oil	10	20	5	10
Fatty Acids (%)				
Linoleic acid	50%	50%	65.8%	65.8%
Oleic acid	28%	25%	17.7%	17.7%
Palmitic acid	10%	10%	8.8%	8.8%
Stearic acid	3.5%	3.5%	3.4%	3.4%
Linolenic acid	9%	9%	4.2%	4.2%
Egg Phosphatides g/100 ml	1.2	1.2	up to 1.2	1.2
Glycerin g/100 ml	2.25	2.25	2.5	2.5
mOsm/L	260	260	276	258
pH	6-8.9	6-8.9	6-9	6-9
Calories/ml	1.1	2	1.1	2

Table I- LIPID EMULSIONS (continued)

Preparation:	LIPOSYN® III 10%	LIPOSYN® III 20%	NUTRILIPID® 10%	NUTRILIPID® 20%
Manufacturer:	Abbott	Abbott	McGaw	McGaw
Components				
Total Concentrn.	10%	20%	10%	20%
Fat Content g/100 ml				
Safflower Oil	---	---	---	---
Soybean Oil	10	20	10	20
Fatty Acids (%)				
Linoleic acid	54.5%	54.5%	49-60%	49-60%
Oleic acid	22.4%	22.4%	21-26%	21-26%
Palmitic acid	10.5%	10.5%	9-13%	9-13%
Stearic acid	4.2%	4.2%	3-5%	3-5%
Linolenic acid	8.3%	8.3%	6-9%	6-9%
Egg Phosphatides g/100 ml	up to 1.2	1.2	1.2	1.2
Glycerin g/100 ml	2.5	2.5	2.21	2.21
mOsm/L	284	292	280	315
pH	6-9	6-9	6-7.9	6-7.9
Calories/ml	1.1	2	1.1	2

In Table I, the vegetable oils, safflower oil and soybean oil (the latter being presently preferred), are each comprised of mixed neutral triglycerides wherein the fatty acid components are as shown. In addition to the oil, the lipid emulsions contain small amounts of egg phosphatides and glycerin, as shown. The oil is emulsified and is in the form of globules which are typically about 0.4 micron in diameter in each lipid emulsion although smaller and larger diameters can be used. The commercial lipid emulsions shown in Table I each contain either 10 or 20 weight percent of the indicated oil.

Typically and illustratively, lipid emulsions that are suitable for use in the practice of this invention can comprise on a 100 weight percent total lipid emulsion basis about 8 to about 25 weight percent lipid oil, about 1 to

about 1.5 weight percent phosphatides, about 1.5 to about 3.5 weight percent glycerin, and the balance up to 100 weight percent being water. However, lipid emulsions or liposomes containing higher or lower oil (lipid) contents and various suspending and stabilizing agents can be used. Preferably, the fat (lipid) is in the form of particles or globules which are below about 0.6 micron in average diameter.

One presently preferred type of lipid emulsion contains on a 100 weight percent total lipid emulsion basis not more than about 20 weight percent soybean oil, about 1.2 weight percent egg phosphatides, about 2.5 weight percent glycerin, and the balance up to 100 weight percent is water.

Particularly when the lipid emulsion is to be used as a vehicle or carrier for AFP, the lipid component and other non-aqueous components should be chosen so that the AFP solution stability is maximized or any tendency for AFP to denature in the presence of the lipid is minimized. It is preferred to avoid or minimize storage of solutions of AFP (whether or not complexed) in lipid emulsion.

(g) The Compositions of AA/PUFA in LE

Compositions of the invention comprised of dissolved AA/PUFA complexes (with or without AFP) in LEs can be prepared by various procedures. For composition stability reasons, and to avoid AFP denaturing, it is presently greatly preferred to have no AFP present in an LE solution (whether or not the AFP is in complexed form). Thus, presently preferred compositions of this invention comprise a complex of AA/PUFA dissolved in an LE with no AFP being present in either free or complexed form. However, solutions of complexes of AA/PUFA/AFP in LEs can evidently be prepared but preferably such solutions are not stored

before administration to avoid AFP denaturing or stability problems.

Typically, a composition of this invention can contain about 0.4 to about 1.5 mg per ml (or about 40 to about 150 weight percent on a total composition weight basis) of AA/PUFA complex (independently of whether or not AFP is present), and preferably about 0.6 to about 1.5 mg per ml, although larger and smaller amounts of AA/PUFA complex dissolved in LE can be used. It is presently more preferred to employ compositions containing at least about 1 mg per ml of AA/PUFA complex (whether or not AFP is present) and not more than about 2 mg per ml of lipid.

In one composition preparation procedure, an ethanol solution of an AA/PUFA complex (without AFP) is injected slowly into the LE while the mixture is rapidly stirred, thereby providing a concentration of the complex dissolved in the LE which preferably is in the range of about 0.6 to about 1.5 mg per ml of LE (or about 60 to about 150 weight percent of complex on a 100 weight percent total solution basis).

In another preparation procedure, an ethanol solution of the AA/PUFA complex is preliminarily added to and dissolved in the main lipid component of the contemplated lipid emulsion. The lipid component can be an oil, such as safflower, soybean, or the like. The addition and dissolution are accomplished before formation of the emulsion.

The ethanol may subsequently be removed from such oil solution or LE solution by evaporation under reduced pressure. The lipid solution is then emulsified into water by the conventional procedure.

A resulting LE solution (which comprises a composition of this invention) typically and preferably

contains about 0.6 to about 1.5 mg/ml of AA/PUFA complex although larger and smaller amounts of the complex can be present. If not removed, or if only partially removed from the product solution, the ethanol can be present in a product LE solution in an amount that can conveniently range up to about 5 weight percent and that typically ranges from about 3 to about 8 weight percent thereof on a total 100 weight percent product basis.

In another preparation procedure, an AA/PUFA complex (with or without AFP) is dissolved in a lipid oil preferably using a lipid oil which has minimal or slowly acting effect upon AFP (if AFP is present). The resulting solution is then mixed with phosphatides, glycerol and water and sonicated to provide a lipid emulsion. It is preferred that such an emulsion be promptly used (administered) if AFP is present.

Regardless of the method of preparation, the content (i.e., quantity) of an AA/PUFA complex that is dissolved in the LE and the content of AFP (if present) can each vary widely. Each can be present in a larger or smaller amount than indicated in the above ranges, if desired.

Presently preferred product compositions are those wherein the AA in the AA/PUFA complex is daunomycin, and the PUFA is arachidonic acid.

If desired, mixtures of different complexes of particular AAs with particular PUFAs (whether or not AFP is present) can be incorporated into a given LE. Various AAs such as disclosed herein can be complexed as described herein with various PUFAs as also disclosed herein.

The product compositions of this invention comprised of AA/PUFA in LE characteristically display excellent shelf life and storage characteristics. These

compositions are useful for the intravascular treatment in mammals, particularly man, of neoplasms, especially AFP absorbing tumor cells whether or not such cells produce AFP. The compositions are especially useful for the destruction of hepatoma cells and of leukemia cells particularly when used in combination with AFP.

(i) Methods of Use

In accord with the present invention, a method is provided for treating neoplastic tissue, especially tumor cells which take up AFP, existing in a mammal, especially including humans. The method comprises administering intravascularly, and preferably intravenously, a composition of this invention. Such a composition, as described above, utilizes at least one AA/PUFA complex dissolved in LE. The presently preferred compositions for use in practicing the method of this invention are as described above. The concentration of the AA/PUFA complex (whether or not AFP is present) in the LE is preferably at least about 0.6 mg per ml of AA/PUFA complex (whether or not AFP is present).

The intravascular dose rate of AA/PUFA complexes according to the method of this invention is preferably chosen so as to be relatively high. Preferably, the dose rate of a composition of this invention based on body weight is at least sufficient to be cytotoxic to substantially all the neoplastic tissue (especially the AFP absorbing neoplastic tissue) that is estimated to be present in the mammal being treated (preferably a human patient). Such a relatively high dose rate of administration does not appear to cause or to result in the side effects that are known to be associated with the use of a free AA. Thus, the characteristically relatively fast-growing tumor cells of neoplastic tissue are destroyed without injuring

appreciably the normal cells or tissue of the treated mammal.

The dose rate, in addition to being related to mammalian body weight, should preferably also be related to the duration of time over which a given dose of a composition of this invention is administered to the mammal involved. In general, relatively short dose administration times are preferred.

While a clinically effective dose rate can vary, based on presently available information, it now appears that, for purposes of treating a mammal (such as man) having AFP absorbing or producing neoplastic tissue, a suitable average daily dose rate for an AA/PUFA complex lies in the range of about 0.5 to about 1.5 mg/kg (milligrams per kilogram) of body weight per 24 hour day. The lipid emulsion composition used for administration of such a dose rate preferably contains at least about 0.4 mg per ml of dissolved AA/PUFA complex in LE and preferably at least about 0.6 mg per ml.

Thus, for example, a human adult having a body weight of about 68 kg can receive an average dose of about 50 to about 100 mg of AA/PUFA complex per day (or about 0.7 to about 1.5 mg/kg per 24 hour period). However, as those skilled in the art will appreciate, the average daily dose rate can be higher or lower than a rate in this exemplary range.

While such an average dose rate can be achieved in an adult patient by daily administration of an AA/PUFA complex, it now appears that approximately equivalent results can be achieved by administering the complex at spaced intervals, such as every other day, or every third day. However, it presently appears that administration intervals between doses can be spaced from one another. For

example, a spacing of not more than about three or four days may be desirable. Thus, the foregoing average daily dose rate is maintained by at least two approximately equally spaced doses per 7-day week. In accord with the present invention, all such dose rates are administered with the AA/PUFA complex being in the LE solution described herein at the foregoing minimal concentration level.

Such an average daily dose rate is entirely consistent with the dose rates which have previously been established for the administration of intravenous aqueous fat (i.e., lipid) emulsions. Thus, lipid emulsions such as those commercially available under the trademark "Liposyn" are now believed to be illustrative and also representative of such emulsions generally. For example, "Liposyn" emulsions which contain either 10% or 20% emulsified fat particles, according to the manufacturer, Abbott Laboratories, can be administered at an infusion rate of up to about 500 ml over a period of about 4 to about 6 hours (which corresponds to a rate of about 1.30 to about 2.10 ml/minute). These lipid emulsions are supplied in various sized units, i.e., 500 ml. The total dose of lipid should not exceed about 1.5 gms/kg of body weight. This manufacturer recommends an initial LE infusion rate for the first 15 minutes of 1.0 ml/minute for "Liposyn" 10% and 0.5 ml/minute for "Liposyn" 20%. Then, if no adverse effects are observed during this initial infusion, the rate can be increased to that indicated above.

Such lipid emulsion infusion rates are now believed to be also suitable for use with the lipid emulsion solutions of AA/PUFA (whether or not AFP is incorporated) as described herein. Thus, for either a 10% or a 20% lipid content lipid emulsion which contains 0.6 mg/ml of AA/PUFA, a complex dose rate of about 1.34 ml/minute for LE solution

produces a dose rate of AA/PUFA of about 0.8 mg/minute, while a complex dose rate of about 2 ml/minute for a LE solution produces a dose rate of AA/PUFA of about 1.2 mg/minute. Similarly, for either a 10% or a 20% lipid content lipid emulsion which contains about 1.5 mg/ml of AA/PUFA, a complex dose rate of about 1.3 ml/minute for LE solution produces a dose rate of AA/PUFA of about 2 mg/minute while a complex dose rate of about 2 ml/minute for LE solution produces a dose rate of AA/PUFA of about 3 mg/minute. A complex dose rate in the range of about 0.8 mg/minute to about 3 mg/minute is thereby achieved.

Thus, for example, a human adult patient having a body weight of about 68 kg receives a dose of about 50 mg/day of AA/PUFA when about 80 to about 85 ml of an LE solution of AA/PUFA complex have been administered which solution contains about 0.6 mg/ml of AA/PUFA, and a dose of about 100 mg/day of AA/PUFA when about 167 ml of such an LE solution have been administered.

Similarly, when a human adult patient receives a dose of about 50 mg/day of AA/PUFA using an LE solution which contains about 1.5 mg/ml of AA/PUFA, about 33 ml of the solution is administered; and a dose of about 100 mg/day of AA/PUFA using such an LE solution is achieved when about 65 to 70 ml are administered. Hence, the per diem dose rate of intravenously administered LE in an LE solution of AA/PUFA is well below the maximum dose rate as indicated above for LE intravenously administered alone.

Continuing the same example, if administration of AA/PUFA in LE solution is carried out on such an adult human patient on alternate days, then each administration would involve twice the per diem average dose level or about 100 to about 200 mg of AA/PUFA. Thus, to achieve this daily average dose level, for a 0.6 mg/ml LE solution of AA/PUFA,

the amount of solution administered would be in the range of about 167 to about 335 ml, and for a 1.5 mg/ml LE solution of AA/PUFA, the amount of LE solution administered would be in the range of about 70 to about 135 ml. For administration of AA/PUFA to such an adult human patient on every third day, one would administer for a 0.6 mg/ml LE solution, an amount of solution that is in the range of about 167 ml to about 335 ml, and for a 1.5 mg/ml LE solution, an amount that is in the range of about 70 ml to about 135 ml. Thus, the spaced intravenous doses of AA/PUFA in LE solution are made within the dose rates that have been previously established for intravenous aqueous fat (i.e., lipid) emulsions.

It appears, based on presently available information, that average daily dose rates for an AA/PUFA complex dissolved in LE as described herein which are, for example, in the range of about 0.5 to about 1.5 mg/kg of adult body weight can be administered repeatedly without any significant patient toxicity. Repeated doses to a patient may be necessary or desirable for purposes of achieving substantial destruction or complete control of the neoplasm involved. A present preference is to limit the total dose of LE to a patient to not more than about 500 ml/day or about 1.5 mg/lipid/kg.

The administration methods of this invention involving LE solutions of AA/PUFA complexes are particularly well suited for the treatment of AFP-producing hepatoma cells in human patients. The serum AFP present in such a patient evidently complexes with the administered AA/PUFA complex, and the resulting three component AA/PUFA/AFP complex circulates to the tumor cells and is taken up through the walls of the tumor cells.

In further accord with the present invention, neoplastic tissue is treated by administering to a mammal as described herein a composition of this invention comprised of AA/PUFA complex (whether or not including complexed AFP) concurrently with a solution (preferably aqueous) of AFP. Such an administration procedure is suited for treatment of tumor cells which absorb or take up AFP (including both tumor cells which produce AFP and tumor cells which do not produce AFP). The procedure is particularly well suited for treatment of tumor cells which absorb or take up AFP but which do not produce AFP.

Such a concurrent administration of dissolved AA/PUFA complex with dissolved AFP permits the in vivo formation of a three component complex of AA/PUFA/AFP. This complex is then taken up by tumor cells which take up AFP.

Also, such a concurrent administration procedure with the AFP being an aqueous solution avoids the potential problems of AFP denaturing by common lipids, or by alcohols, such as ethanol or of shelf life instability. The AFP can be, and preferably is, prepared in aqueous solution form just prior to administration. The present preference to prepare such an aqueous solution from powdered AFP, where the powdered AFP is prepared in powdered form by purification and lyophilization, such as described herein.

The term "concurrently" or "concurrent", as used herein in relation to administration of an AA/PUFA complex and AFP (whether or not the AFP is complexed), includes reference to:

(a) administering an LE solution of an AA/PUFA complex first followed by administration of an aqueous solution of AFP,

(b) administering an LE solution of an AA/PUFA complex after administration of an aqueous solution of AFP,

(c) administering a mixture of both an LE solution of an AA/PUFA complex and an aqueous solution of AFP, and/or

(d) administering a dissolved complex of AA/PUFA/AFP.

Administration of a preformed complex of AA/PUFA/AFP is believed to be possible in one mode of the method of this invention. However, it now appears to be difficult to prepare such a three-component complex in a concentrated liquid solution form such as is desired for use in the practice of the method of this invention. Because of what are now believed to be the possible stability and denaturing characteristics associated with AFP in the presence of nonaqueous solvents, it now appears that the presently provided preferred safe and practical concurrent administration method for achieving tumor cell treatment with in situ formation of the three-component complex may be unique. The LE carrier for an AA/PUFA complex appears to provide an excellent transfer medium so that the three component complex can be produced in vivo safely and effectively. Also, the LE permits achievement of high AA/PUFA complex concentrations that can be used with high aqueous solution concentrations of AFP.

In concurrent administration, the foregoing procedures and dose rates for administering solutions of AA/PUFA in LE are utilized. The exact dose rate for an intravascularly administered AFP aqueous solution is preferably correlated with the exact dose rate for such an LE solution. As a convenient estimate, a mole ratio of AA/PUFA complex to AFP that is in the range of about 1:1 to about 1:4 can be sought in vivo after the AA/PUFA and the AFP have been administered. Typically, the dose rate for an

AFP solution falls in the range of about 50 to about 125 mg/kg of mammalian body weight per 24 hour day, but larger and smaller such rates can be used. As a present preference, after concurrent administration, the in vivo mole ratio of the administered AA/PUFA complex to the AFP falls in the (calculated) range of about 1:1 to 1:0.5, but such mole ratios are illustrative only, and larger and smaller such mole ratios can be employed without departing from the spirit and scope of the present invention. A presently employed and presently most preferred such weight ratio is about 1-8.5 (as used in the following Examples).

An administered AA/PUFA complex in LE solution form is now believed to be (based on available data) substantially completely taken up and removed from the treated animal's blood stream within about several hours after administration. An administered AFP in solution is believed to be taken up over a longer time interval. Preferably, the concurrent administration is promptly carried out well within such a several hour time frame when successive administration of a separate AFP aqueous solution and a separate AA/PUFA LE solution is being utilized. Most preferably, not more than about 5 to about 15 minutes elapses between such successive administrations. A separately prepared aqueous AFP solution can be preliminarily admixed with a separately prepared LE solution of AA/PUFA to prepare a starting mixed solution wherein the mole ratio of AA/PUFA complex to AFP is within the foregoing range. However, it is now preferred to complete concurrent administration of such a mixture promptly after its preparation, thereby to avoid or minimize possible problems with AFP stability or denaturing.

The exact pharmacological and biochemical mechanisms involved in the practice of the method of this

invention are not currently known. However, as indicated above, it is now believed that, after a dissolved AA/PUFA complex enters the bloodstream of a patient afflicted with neoplastic tissue which absorbs AFP, from a lipid emulsion (LE), AFP that is present in the patient's blood serum (whether produced in vivo from body sources or administered intravascularly) bonds to the PUFA of the AA/PUFA complex to form a tumor cell targeted complex of AA/PUFA/AFP.

Evidently, the LE functions as a carrier or transfer agent, and, most likely, based on present evidence, it does not enter the neoplastic tissue cells. The AA/PUFA complex evidently leaves the LE sometime after intravascular administration and this two-component complex of AA/PUFA is taken up by, or bonded to, AFP circulating in the blood serum of the patient.

The resulting in situ formed AA/PUFA/AFP complex then circulates in the blood stream to the location of the target AFP fetoprotein-absorbing neoplastic tissue where the neoplastic tissue cells preferentially take up the AA/PUFA/AFP complex resulting in the destruction of those cells. Since normal cells are generally slower replicating than neoplastic tissue cells, and since the AA/PUFA complex and the AA/PUFA/AFP complex have substantially less toxicity towards normal cells than the AA alone, the normal cells are not appreciably damaged during the preferably relatively brief period of complex administration to a patient. However, since the dose rate for an AA/PUFA complex is preferably relatively high, it is probable that circulating AFP will be bound by the two-component AA/PUFA complex. A maximum attack on the neoplastic tissue is believed to be made possible following the administration of the complex.

Based on presently available evidence, it is believed that, after administration of an AA/PUFA complex

(in LE), a portion of the complex is taken up by (normal) reticuloendothelial cells. Some depression of white and red cell levels and of growth in rats is seen particularly at high dose rates. However, these changes are reversed after cessation of administration of the complex. This indicates that no irreversible damage to the bone marrow or to other organs has occurred.

Because of the apparent effectiveness in treating neoplastic tissue, especially AFP-absorbing neoplastic tissue, that is achieved by using the compositions and methods of this invention, it is possible that a synergistic coaction exists between an AA/PUFA complex and an LE with regard to the capacity for an inventive solution of an AA/PUFA complex in LE to treat AFP-absorbing neoplastic tissue compared to the ability of solutions of the same complexes in alternative carriers to treat the same neoplasms. The surprisingly high concentrations of complexes of the AA/PUFA type as described herein that can be achieved in an LE are not known to exist for other carriers. Such high concentration capacity makes possible or substantially contributes to the efficacy and safety of the new and improved preferred treatment methods of this invention.

In general, administration of an LE solution of an AA/PUFA complex with dissolved AFP is preferably continued for a time period which is sufficient to treat AFP producing neoplastic tissue, such as AFP-producing hepatic tissue. This treatment is insufficient to produce long term cytotoxic, or other undesirable side effects, in normal mammal tissue or organs, such as the undesirable effects that are known to be associated with the use of free AA. Although an AA/PUFA complex appears to be safe, prolonged use thereof could produce undesirable AA-associated side

effects. It is therefore presently preferred, on the basis of limited information, to administer such an LE solution for a total continuous time period that is not longer than about 12 days.

It is presently preferred to practice the methodology of this invention using a single dose pharmaceutical system. Such a system comprises in combination (a) a container (or chamber) containing a single dosage of a α-fetoprotein in the form of a water soluble powder, and (b) a container (or chamber) containing a single dosage of a liquid composition comprising a complex of an anthracycline antibiotic with a polyunsaturated fatty acid which is dissolved in a lipid emulsion. Optionally, a container (or chamber) containing a single dosage of an aqueous sterile carrier liquid can be used in which the powdered AFP can be dissolved before administration. Preferably this system further includes a volume of distilled water wherein the powdered AFP can be dissolved to make up a solution for immediate administration. The dose levels of the AFP and the AA/PUFA complex and the respective concentrations of each in their respective solvent carriers can be within the ranges described herein.

Embodiments

The present invention is further illustrated with reference to the following Examples:

Example 1. Preparation of a Solution of AA/PUFA Complex in LE

A daunomycin complex with arachidonic acid (1:1 mole ratio) is prepared as described by Deutsch et al. in the foregoing Cancer Research article.

The product AA/PUFA complex which has a mole ratio of daunomycin to arachidonic acid of about 1:1 is dissolved in ethanol to provide a solution of about 8 to about 15 mg

of the daunomycin-arachidonic acid complex per ml of ethanol.

This ethanol solution is slowly added with vigorous stirring to an "Intralipid 20%" lipid emulsion (composition as shown in Table I above) obtained from Kabi Pharmacia, Inc. (Clayton, NC). The complex dissolves in the lipid emulsion. The resulting lipid emulsion solution has a dissolved ethanol concentration of about 3 to about 5 weight percent (based on 100 weight percent total lipid emulsion solution weight) and contains about 0.6 to about 1.2 mg/ml of dissolved daunomycin-arachidonic acid complex (based on the total volume of the resulting solution).

Example 2. Treatment of Hepatoma in Rats

The following solutions were used:

(1) 0.7 ml per rat aqueous phosphate buffered saline, pH 7.4;

(2) "Intralipid 20%" (0.7 ml per rat);

(3) 200 μg per rat free daunomycin dissolved in 0.7 ml "Intralipid 20%"; and

(4) a solution of Example 1 (0.285 mg/ml of daunomycin/arachidonic acid complex dissolved in "Intralipid 20%" (equivalent to 200 μg daunomycin per 0.7 ml per rat).

Donryu rats weighing about 150 grams each were given 10,000 AH66 hepatoma cells administered by injection intravenously (via the tail vein) at day one of this evaluation.

Hepatoma tumor cells characteristically produce and introduce alpha-fetoprotein into circulating mammalian blood serum and such cells also absorb alpha-fetoprotein from this blood serum.

Three days later, the members of respective groups of the rats (5 rats per group) were each injected intravenously with 0.7 ml (via the tail vein) of one of the solutions shown above. The dose rate per rat for the daunomycin/arachidonic acid complex was about 1.3 mg/kg/day. These injections were repeated at two day intervals, and a total of five injections were given to each rat. The results are shown below in Table II:

TABLE II

Comparative Effect of Daunomycin/Arachidonic Acid Complex In "Intralipid 20%" on Rat Hepatoma

Group*	Substance Injected	Days Survival Each Rat*	Mean Days Survival
1.	0.7 ml Phosphate Buffered Saline, pH 7.4	17, 18, 21, 23, 24	20.6
2.	0.7 ml/rat Intralipid 20%	21, 22, 25, 27, 27	24.4
3.	200 μg per rat free dissolved Daunomycin in 0.7 ml Intralipid 20%	27, 32, 33, 37, 38	33.4
4.	Daunomycin-Arachidonic Acid Complex** in 0.7 ml Intralipid 20%	40, 43, 51 60, 60***	

Table II Footnotes:

* Each injected group included five rats.

** Equivalent to 200 μg of Daunomycin per rat.

*** The two rats surviving 60 days were killed and autopsied. No tumors were found in these animals.

The results shown in Table II demonstrate that a solution of daunomycin/arachidonic acid complex in a lipid

emulsion is effective for treating AFP-producing and AFP-absorbing hepatoma cells in rats.

Example 3. Treatment of Leukemia in Rats

The following solutions were used:

1. Saline (0.15 molar sodium chloride; 1.0 ml/rat)
2. AFP (500 μg/rat) in 1.0 ml/rat of saline
3. 1.0 ml/rat of "Intralipid 20%"
4. AFP (500 μg/rat) in 1.0 ml/rat of "Intralipid 20%"
5. Daunomycin (100 μg/rat in 1.0 ml/rat of "Intralipid 20%"
6. AFP (500 μg/rat) plus daunomycin (100 μg/rat) in 1.0 ml/rat of "Intralipid 20"
7. Daunomycin/arachidonic acid complex (100 μg) in 1.0 ml "Intralipid 20"
8. AFP (500 μg) plus daunomycin/arachidonic acid complex (100 μg) in 1.0 ml "Intralipid 20"

Donryu rats weighing about 150 grams each were given 10^6 leukemia cells (DBLA-1) administered by injection intravenously (via the tail vein) at day one of this evaluation.

Leukemia tumor cells characteristically do not produce alpha-fetoprotein, but they do absorb alpha-fetoprotein from circulating blood serum.

Two days later the members of respective groups of rats (5 rats per group) were each injected intravenously with 1.0 ml (via the tail vein) of one of each of the solutions shown above. The dose rate per rat for the daunomycin/arachidonic acid complex was about 0.7 mg/kg/

day which was equivalent to the dose of 100 μg per rat of daunomycin. The mole ratio of daunomycin to AFP was about 25. These injections were repeated at two day intervals and a total of five injections were given to each rat. The results are shown below in Table III:

TABLE III

Comparative Effect of Daunomycin/Arachidonic Acid Complex in "Intralipid 20%" on Leukemia With And Without The Presence Of Injected Alpha-Fetoprotein

Solution Ident. No.	Rat Mean Days Survival In Each Group
1	18.8 (± 0.0)
2	21.1 (± 1.2)
3	18.2 (± 1.2)
4	18.6 (± 1.5)
5	30.8 (± 2.1)
6	30.6 (± 1.7)
7	28.0 (± 1.7)
8	38.2 (± 2.3)

The results shown in Table III demonstrate that a solution of daunomycin/arachidonic acid complex in a lipid emulsion plus AFP is effective for treating AFP-absorbing leukemia cells in rats.

Example 4. Treatment of Leukemia in Rats

The following solutions were used:

1. Phosphate buffered saline (1.0 ml/rat)
2. Lipid emulsion ("Intralipid 20%") (1.0 ml/rat)

3. AFP (5 mg/rat) in 1.0 ml "Intralipid 20%"/rat

4. Daunomycin (100 μg/rat) in 1.0 ml "Intralipid 20%"/rat

5. Daunomycin/arachidonic acid C20:4 (100 μg/rat) in 1.0 ml "Intralipid 20%"/rat

6. Daunomycin/arachidonic acid C20:4 (100 μg/rat) plus AFP (5 mg/rat) in 1.0 ml "Intralipid 20%"/rat

The protocol was the same as in Example 3. The results are shown in Table IV below:

TABLE IV

Comparative Effect of Daunomycin/Arachidonic Acid Complex in "Intralipid 20%" on Leukemia With And Without The Presence Of Injected Alpha-Fetoprotein

Solution Ident. No.	Days Survival Each Rat	Mean Survival Days
1	16, 18, 20, 22, 23	(19.8, ± 1.3)
2	14, 16, 17, 20, 22	(17.8, ± 1.4)
3	15, 16, 18, 21, 24	(18.8, ± 1.7)
4	24, 29, 33, 36, 40	(32.4, ± 2.8)
5	23, 26, 31, 34, 37	(30.2, ± 2.6)
6	38, 42, 43, 47, 54	(44.8, ± 2.7)

The results shown in Table IV are comparable to, and confirm, the results shown in Table III (above).

Example 5. Treatment of Hepatoma in Rats

The solutions of Example 2 are again employed in a repeat of the same protocol except that here the dose of solution per rat is reduced to 0.2 ml from 0.7 ml. The total daunomycin administered per rat was 500 μg. The results are shown below in Table V:

TABLE V

Comparative Effect of Daunomycin/Arachidonic Acid Complex in "Intralipid 20%" on Rat Hepatoma

Group	Experimental Condition	Days Surviving	Mean Days Survival
1	0.2 ml PBS	18, 19, 22, 24, 25	(21.6)
2	0.2 ml Lipid Emulsion	20, 21, 24, 25, 25	(23.0)
3	100 μg free Daunomycin in 0.2 ml Emulsion	25, 28, 31, 32, 34	(30.0)
4	100 μg Dauno. -C20:4 in 0.2 ml Lipid Emulsion	39, 42, 45, 52, 54	(46.4)

Example 6. Preparation of a Solution of AA/PUFA Complex in LE

A complex of daunomycin with arachidonic acid is prepared as described in Example 1 and is separated as a dry powder by lyophilization and taken up in ethanol.

The resulting complex is dissolved in soybean oil to provide a solution of about 5 mg of daunomycin/ arachidonic acid per ml of total oil. This oil solution is mixed with phosphatides, glycerol and water and then sonicated to provide a lipid emulsion containing about 20% lipid and about 1 mg/ml of the complex of daunomycin/ arachidonic acid.

Example 7. Solution of AA/PUFA and AFP

To the solution of Example 7 is added a solution of weight percent AFP in aqueous 0.15 molar sodium chloride (saline) with gentle stirring until a uniform mixture is produced which is suitable for intravenous administration.

Example 8. Preparation of AFP

A sample of AFP is purified by conventional methods using human hepatoma cell culture media, umbilical cord blood or abortion fluids as starting material. A 1% to 5% solution of this protein in water is placed into a suitably sized ampoule, rapidly frozen.

The solution in the ampoule is then rapidly frozen in a cold bath of acetone and solid carbon dioxide. The container is held at a 45 degree angle and slowly rotated in the cold bath to allow the solution to freeze. The solution is frozen along the container wall in the form of a shell.

The ampoule containing the frozen solution is then connected to a lyophilizer. The seal between the lyophilizer and the ampoule interior is broken to allow maintenance of proper vacuum in the ampoule. A cold trap condenses the water vapor associated with the protein during lyophilization.

During lyophilization, the sample is left undisturbed until all of the ice has evaporated. After this

process is completed, the sample may be sealed under vacuum and held so until used. The dried white powder is readily soluble in water or isotonic physiological buffers and an approximately 10 mg per ml solution of the AFP is prepared for administration purposes immediately prior to use.

Example 9. Evaluation of Toxicity of Free Daunomycin in Lipid Emulsion

The following solutions were used:

1. "Intralipid 20%" (2 ml/rat)
2. Daunomycin (500 μg per rat) in 2 ml "Intralipid 20%"/rat

The protocol was as follows: Using two groups of five Donryu rats each, intraperitoneal injections of each solution with a separate rat group were started at day zero. A total of ten injections were given to each in two day spaced intervals. The starting and terminal animal weight, erythrocyte and white blood cell levels were determined and recorded. Results are shown in Table VI below:

TABLE VI

Toxicity of Free Daunomycin

	Controls (2 ml LE)			500 μg Daunomycin in LE		
Day	Wt.(gms)	r.b.c.	w.b.c.	Wt.(gms)	r.b.c.	w.b.c.
0	100	6.05	9340	100	6.02	9500
21	166	5.94	9420	87*	3.17	2233

* Mean of 3 surviving animals after 9 injections. Average survival days 22 (17-27).

The results as shown in Table VI show the daunomycin to be toxic.

Example 10. Evaluation of Toxicity of Daunomycin - Arachidonic Acid Complex in Lipid Emulsion

The following solutions were used:

1. "Intralipid 20%" (2 ml/rat)
2. Daunomycin/arachidonic acid complex (200 μg per rat) in 2 ml "Intralipid 20%"
3. Daunomycin/arachidonic acid complex (500 μg per rat) in 2 ml "Intralipid 20%"
4. Daunomycin/arachidonic acid complex (1000 μg per rat) in 2 ml "Intralipid 20%"

The protocol was the same as in the preceding Example. The starting, 28-day and 56-day animal weight erythrocyte and white blood cell levels were determined and recorded. Results are shown in Table VII below:

TABLE VII

Toxicity of Daunomycin-C20:4 Complex

Daunomycin-C20:4 in Lipid Emulsion

	Lipid Emulsion			200 μg		
Day	Wt.	r.b.c.	w.b.c.	Wt.	r.b.c.	w.b.c.
0	100	6.05	9340	100	5.99	8880
28	184	6.01	9200	183	5.78	8060
56	244	6.19	9600	241	5.84	9060

	500 μg			1000 μg		
Day	Wt.	r.b.c.	w.b.c.	Wt.	r.b.c.	w.b.c.
0	99	5.89	8940	99	5.91	8840
28	159	4.82	7080	126	4.77	5260
56	227	5.91	8560	205	5.37	7460

The results show that even at the 1000 μg level of administration, little toxicity results and following the last injection at day 18, weights, r.b.c. and w.b.c. levels return to normal.

Example 11. Treatment of Hepatoma in Rats

To evaluate a different AA, idarubicin was employed instead of daunorubicin.

1. Phosphate buffered saline (0.7 ml per rat)
2. Lipid Emulsion ("Intralipid 20%") (0.7 ml per rat)
3. Idarubicin (200 μg per rat) in 0.7 ml per rat of "Intralipid 20%"
4. Idarubicin/arachidonic acid complex (200 μg per rat) in 0.7 ml per rat of "Intralipid 20%"
5. Daunomycin/arachidonic acid complex (200 μg per rat) in 0.7 ml per rat of "Intralipid 20%"

The protocol used was the same as in Example 2. The results are shown in Table VIII below:

TABLE VIII
Effect of Idarubicin-C20:4 on AH66 Rat Hepatoma

Group	Experimental Conditions	Days Survival	Mean Days
1	Phosphate Buffered Saline	16, 19, 20, 21, 23	(19.8, ± 1.2)
2	Lipid Emulsion	19, 21, 22, 24, 27	(22.6, ± 1.4)
3	Idarubicin (200 μg in Lipid Emulsion	23, 25, 29, 32, 35	(28.8, ± 2.2)
4	Idarubicin/Arachidonic Acid Complex (200 μg) in Lipid Emulsion	37, 41, 45, 49, 55	(45.4, ± 3.1)
5	Daunomycin/Arachidonic Acid Complex (200 μg) in Lipid Emulsion	42, 45, 47, 53, 58	(49.0, ± 2.9)

The results as shown in Table VIII show that idarubicin/arachidonic acid complexes are similar to, but not as effective as, daunomycin/arachidonic acid complexes in ability to control hepatomas.

Variations, modifications, alterations and additional embodiments will be apparent to those skilled in the art from the foregoing description without departing from the scope of this invention.

WHAT IS CLAIMED IS:

1. A composition for the intravascular treatment of α-fetoprotein-absorbing neoplastic tissue in a mammal comprising;

(a) at least one complex that is selected from the group consisting of

(1) complexes of an anthracycline antibiotic with a polyunsaturated fatty acid wherein the mole ratio of said anthracycline antibiotic to said polyunsaturated fatty acid is about 1:1; and

(2) complexes of an anthracycline antibiotic with a polyunsaturated fatty acid and also with an α-fetoprotein wherein the mole ratio of said anthracycline antibiotic to said polyunsaturated acid to said α-fetoprotein is in the range of about 1:1:1 to about 1:1:2;

(b) α-fetoprotein;

(c) said complex being dissolved in a lipid emulsion;

(d) said α-fetoprotein being dissolved in at least one solvent selected from the group consisting of lipid emulsion and water;

(e) said anthracycline antibiotic comprises a tetracycline aglycone which is bonded through an ether linkage to a basic amino sugar;

(f) said polyunsaturated acid contains about 18 to 24 carbon atoms per molecule, at least three double bonds between respective adjacent carbon atom pairs per molecule, and a single terminal carboxyl group per molecule;

(g) said α-fetoprotein having an amino acid sequence that is characteristically mammalian; and

(h) said lipid emulsion comprises on a 100 weight percent total lipid emulsion basis about 8 to about 25 weight percent vegetable oil, about 1 to about 1.5 weight percent phosphatides, and about 1.5 to about 3.5

weight percent glycerin, and the balance up to 100 weight percent being water.

2. The composition of claim 1 wherein said complex comprises said anthracycline antibiotic and said polyunsaturated fatty acid.

3. The composition of claim 1 wherein the amount of said complex that is dissolved in said lipid emulsion is in the range of about 0.4 to about 1.5 milligrams per milliliter of said lipid emulsion based on a complex of said anthracycline antibiotic and said polyunsaturated acid.

4. The composition of claim 1 wherein said anthracycline antibiotic is selected from the group consisting of daunomycin, doxorubicin, idarubicin and pirarubicin.

5. The composition of claim 1 wherein said polyunsaturated acid contains at least about 20 carbon atoms per molecule and at least four double bonds per molecule.

6. The composition of claim 4 wherein said polyunsaturated acid is selected from the group consisting of arachidonic acid and docosahexaneoic acid.

7. The composition of claim 1 wherein said α-fetoprotein is human α-fetoprotein.

8. The composition of claim 1 which additionally contains dissolved uncomplexed α-fetoprotein.

9. The composition of claim 1 wherein, in said complex, said anthracycline antibiotic is daunomycin, said polyunsaturated acid is arachidonic acid, and said α-fetoprotein, when present, is human α-fetoprotein.

10. The composition of claim 1 wherein said lipid emulsion comprises on a 100 weight percent total lipid emulsion basis about 10 to about 20 weight percent soybean oil, about 1.2 weight percent egg phosphatides, about 2.5 weight percent glycerin, and the balance up to 100 weight percent being water.

11. α-Fetoprotein in purified powdered form.

12. An aqueous solution of α-fetoprotein.

13. The solution of claim 12 which contains about 0.15 molar dissolved sodium chloride and which contains about 10 to about 20 milligrams per milliliter of said alpha-fetoprotein.

14. A method for treating neoplastic tissue existing in a mammal comprising administering intravascularly to said mammal a composition of claim 1 in an amount which is at least sufficient to destroy said neoplastic tissue.

15. The method of claim 14 wherein said neoplastic tissue produces and takes up α-fetoprotein.

16. The method of claim 14 wherein said neoplastic tissue takes up α-fetoprotein and said administering is carried out concurrently with the administration of an aqueous solution of α-fetoprotein.

17. The method of claim 14 wherein said mammal is a human being.

18. The method of claim 14 wherein said neoplastic tissue comprises α-fetoprotein-producing hepatoma cells.

19. The method of claim 14 wherein the dose rate of said complex so administered averages about 0.4 to about 1.5 milligrams per kilogram of mammal body weight per day, based on a complex of said anthracycline antibiotic and said polyunsaturated fatty acid.

20. The method of claim 14 wherein said complex comprises an anthracycline antibiotic and a polyunsaturated fatty acid, and the amount of said complex that is dissolved in said lipid emulsion is in the range of about 0.6 to about 1.5 milligrams per milliliter of said lipid emulsion.

21. The method of claim 14 wherein said anthracycline antibiotic is daunomycin; said

α-fetoprotein when present is human α-fetoprotein; said polyunsaturated fatty acid is arachidonic acid; and said lipid emulsion comprises on a 100 weight percent total lipid emulsion basis about 10 to about 20 weight percent soybean oil, about 1.2 weight percent egg phosphatides, about 2.5 weight percent glycerin, and the balance up to 100 weight percent being water.

22. The method of claim 14 wherein said administration of said α-fetoprotein is accomplished after said complex is so administered.

23. The method of claim 14 wherein said administration of said α-fetoprotein is accomplished before said complex is so administered.

24. The method of claim 14 wherein said administration is accomplished concurrently with said administration of said complex and said α-fetoprotein solution is in admixture with said composition.

25. An article of manufacture for use as a single dose pharmaceutical system comprising in combination:

(a) a container containing a single dosage of α-fetoprotein in the form of a water soluble powder; and

(b) a container containing a single dosage of a liquid composition comprising a complex of an anthracycline antibiotic with a polyunsaturated fatty acid which is dissolved in a lipid emulsion.

26. The composition of claim 1 which is in two parts such that a first part comprises a solution of said complex in said lipid emulsion and said complex comprises said anthracycline antibiotic with said polyunsaturated acid and said second part comprises a solution of said α-fetoprotein in an aqueous medium.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/07949

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 31/20, 31/70, 37/00; C07K 3/00
US CL :514/21, 34, 560; 530/380
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/21, 34, 560; 530/380

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,772,590 (Kawata et al) 20 September 1988, col.2, lines 13-69.	1-10 and 14-26
Y	US, A, 5,004,593 (Ames et al) 02 April 1991, col. 2, lines 1-20.	1-10 and 14-26
Y	Cancer Research, volume 43, issued June 1983, H. F. Deutsch et al, "Cytotoxic Effects of Daunomycin-Fatty Acid Complexes on Rat Hepatoma Cells", pages 2668-2672, especially page 2668.	1-26
X	Tumor Biology, Volume 8, issued 1987, H. F. Deutsch et al, "Anthracycline-Polyunsaturated Fatty Acid (PUFA) Complexes with Alpha-Fetoprotein (AFP) and Their Uptake by Tumor Cells", pages 289-290, entire document.	1-26

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

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Name and mailing address of the ISA/
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. NOT APPLICABLE

Authorized officer
ELLI PESELEV

Telephone No. (703) 308-0196